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Description of *Rhodnius marabaensis* sp. n. (Hemiptera, Reduviidade, Triatominae) from Pará State, Brazil

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Abstract

Rhodnius marabaensis **sp. n.** was collected on 12 May 2014 in the Murumurú Environmental Reserve in the city of Marabá, Pará State, Brazil. This study was based on previous consultation of morphological descriptions of 19 *Rhodnius* species and compared to the identification key for the genus *Rhodnius*. The examination included specimens from 18 *Rhodnius* species held in the Brazilian National and International Triatomine Taxonomy Reference Laboratory in the Oswaldo Cruz Institute in Rio de Janeiro, Brazil. The morphological characteristics of the head, thorax, abdomen, genitalia, and eggs have been determined. *Rhodnius prolixus* and *R. robustus* were examined in more detail because the BLAST analysis of a cyt-b sequence shows they are closely related to the new species, which also occurs in the northern region of Brazil. The most notable morphological features that distinguish *R. marabaensis* **sp. n.** are the keel-shaped apex of the head, the length of the second segment of the antennae, the shapes of the prosternum, mesosternum and metasternum, the set of spots on the abdomen, the male genitalia, the posterior and ventral surfaces of the external female genitalia, and the morphological characteristics of the eggs. *Rhodnius jacundaensis* Serra, Serra and Von Atzingen (1980) *nomen nudum* specimens deposited at the Maraba Cultural Center Foundation - MCCF were examined and considered as a synonym of *R. marabaensis* **sp. n.**

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Keywords
Triatominae, *Rhodnius marabaensis* sp. n., new species, Amazon

Introduction

Vectors of the protozoan *Trypanosoma cruzi*, the etiological agent of Chagas disease, include 151 species distributed into 18 genera belonging to the subfamily Triatominae (Galvão 2014, Mendonça et al. 2016). The genus *Rhodnius* includes 19 species (Alevi et al. 2015), of which six were described after the publication of the Lent and Wygodzinsky review (1979): *R. stali* Lent, Jurberg & Galvão, 1993; *R. colombiensis* Mejia, Galvão & Jurberg, 1999; *R. milesi* (Carcavallo, Rocha, Galvão & Jurberg, 2001); *R. zeledoni* Jurberg, Rocha & Galvão, 2009; *R. montenegrensis* Rosa et al., 2012, and *R. barretti* Abad-Franch, Palomeque & Monteiro, 2013. *R. amazonicus* Almeida, Santos & Sposina, 1973 was synonymized with *R. pictipes* by Lent and Wygodzinsky (1979) according a photograph of the holotype, but subsequently it was validated by Bérenger and Pluot-Sigwalt (2002) by morphological study of 19 characters with of the two species.

Among the *Rhodnius* species, only nine are found in the northern region of Brazil: *R. amazonicus*, *R. brethesi*, *R. milesi*, *R. montenegrensis*, *R. neglectus*, *R. paraensis*, *R. pictipes*, *R. robustus*, and *R. stali* (Galvão 2014, Meneguetti et al. 2015).

In May 2014, two *Rhodnius* spp. specimens were collected in Marabá, Pará, Brazil, and compared to the key described by Lent and Wygodzinsky (1979), as well as to previously described *Rhodnius* species, without success. These samples were compared and identified as the same species as *Rhodnius jacundaensis* Serra, Serra & Von Atzingen, 1980 which had been deposited at the Marabá Cultural Center in Pará. *Rhodnius jacundaensis* was mentioned in an abstract presented at the Fourth Annual Brazilian Parasitology Conference in Rio de Janeiro in 1980. According to Article 9 of the International Code of Zoological Nomenclature, however, this new species was not confirmed. As a result, Carcavallo et al. (1998) and Galvão et al. (2003) considered it to be *R. jacundaensis* Serra, Serra & Von Atzingen, 1980) *nomen nudum*. This article describes *Rhodnius marabaensis* sp. n. as the tenth species found in northern Brazil and the twentieth member of this genus (Abad Franch et al. 2013, Galvão 2014, Meneguetti et al. 2014, Alevi et al. 2015).

Materials and methods

Morphological identification and description

The specific description was based on the observation of two adult specimens (one female and one male) collected in a residence in the Murumuru Environmental Reserve in the city of Marabá, Pará, Brazil (coordinates: 10°10'05.1"S and 63°24'09.1"W)

Figure 1. Localization of Marabá- PA where *R. marabaensis* sp. n. specimens were collected (05°21'54"S, 49°07'24"W).

(Fig. 1). The description included 14 males and 14 females deposited at the MCCF and previously characterized as *R. jacundaensis* [*nomen nudum*].

The identification of samples was performed using the dichotomous key by Lent and Wygodzinsky (1979). The study also considered descriptions of *R. amazonicus*, *R. stali*, *R. colombiensis*, *R. milesi*, *R. zeledoni*, *R. montenegrensis*, and *R. barretti* (Almeida et al. 1973, Lent et al. 1999, Mejia et al. 1999, Valente et al. 2001, Jurberg et al. 2009, Rosa et al. 2012, Abad-Franch et al. 2013). *Rhodnius marabaensis* was also compared to specimens of 18 *Rhodnius* species held at the Brazilian National and International Triatomine Taxonomy Reference Laboratory at the Oswaldo Cruz Institute in Rio de Janeiro, Brazil. The only species that was compared only by description was *R. amazonicus*, which is held at the Brazilian National Institute of Amazon Research (INPA).

Genetic identification

After the identification, the mitochondrial gene fragment cytochrome b (cyt-b) was amplified by using suggested primers by Monteiro et al. (2003). The amplified fragments were purified and sequenced in duplicate (forward and reverse). The same haplotype was shown with 693 base pairs (bp). This sequence was evaluated by BLAST (http://www.blast.ncbi.nlm.nih.gov/Blast.cgi) to diagnose the homologous sequences in GenBank. In view of this and of the fact that all three species occur in Northern Brazil (Galvão et al. 2003, Galvão 2014), *R. robustus* and *R. prolixus* were morphologically examined and compared in more detail.

Morphological study

For the comparative morphological study, *R. prolixus* specimens from Araraquara Triatominae Colony (CTA) 074 were used, as were *R. robustus* specimens from CTA085. The specimens were kept in these colonies at the Triatominae Insectarium at the School of Pharmaceutical Sciences of São Paulo State University (UNESP), Araraquara, São Paulo, Brazil. The *R. prolixus* colony had been originally collected in a sylvatic environment in Venezuela on 23 May 1983. The *R. robustus* colony has been maintained since February 1972 using specimens from Peru (Fig. 2).

Optical microscopy and scanning electron microscopy (SEM) were used to compare the morphology of *R. marabaensis* sp. n., *R. prolixus*, and *R. robustus.*The head, the ventral portion of the thorax, the scutellum, and the pygophore were studied using SEM (Figs 3–6, 8). A female of *R. marabaensis* sp. n. that had been collected in May 2014 was used to study the female genitalia, and 13 eggs obtained from its uterus were also analyzed by SEM (Figs 9–13).

Morphometric study

A Leica MZ APO stereoscope and the Motic Images Advanced System version 3.2 were used for the measurements, as well as for the study of *R. marabaensis* sp. n. male genitalia. For the comparative study of the male genitalia of *R. marabaensis* sp. n. and *R. prolixus* and *R. robustus*, the descriptions by Lent and Jurberg (1969) and by Rosa et al. (2012) were used.

The following parameters of both females (15) and males (15) were measured: total length (TL); head length (HL); the length of the four antennal segments (A_1, A_2, A_3, and A_4); the three segments of the proboscis (R_1, R_2, and R_3); the inner distance between the eyes (IE); the outer distance between the eyes (OE); the diameter of the eyes (DE); the maximum width of the abdomen (MWA); the maximum width of the thorax (MWT); and the length, width, and diameter of the opercular opening of the eggs (Lent and Wygodzinsky 1979, Dujardin et al. 1999, Rosa et al. 2000).

Taxonomy

Family Reduviidae Latreille, 1807
Subfamily Triatominae Jeannel, 1919
Genus *Rhodnius* Stål, 1859

***Rhodnius marabaensis* sp. n.**
http://zoobank.org/883B9B62-9E78-4AFF-9518-021593A308A4

Holotype ♂. **BRAZIL: Pará**: Marabá: Reserva Ambiental Murumurú, 10°10'05.1"S, 63°24'09.1"W, 12 May 2014, N. C. B. Von Atzingen, M. B. Furtado, UNESP.

Paratypes. 1 ♀: same data as holotype (UNESP). 14 ♀, 14 ♂ **BRAZIL:** Pará: Jacundá/Jatobal/Marabá,N.C.B. Von Atzingen, Maraba Cultural Center Foundation – MCCF.

Synonym. *Rhodnius jacundaensis* Serra, Serra and Von Atzingen (1980) [*nomen nudum*].

Etymology. The name *Rhodnius marabaensis* was chosen because this species was found in the city of Marabá, Pará, Brazil.

Diagnosis. The most notable morphological features that distinguish *R. marabaensis* sp. n. are the keel-shaped apex of the head, this feature is not accentuated in *R. prolixus* or *R. robustus* ; the second antennal segment of *R. marabaensis* sp. n. is 10.3 times larger than the first; in *R. prolixus*, it is 6.2 times larger, and in *R. robustus* it is 8.3 times larger. The prosternum has a longer and more clearly shaped stridulatory sulcus relative to those of *R. prolixus* and *R. robustus*. In *R. marabaensis* sp. n. the transverse carinae that border the mesosternum and the metasternum are elevated and prominent, and possess convex curvature in the central portion. In *R. prolixus*, they are less elevated and prominent, and in *R. robustus* they are interrupted in the central portion. The set of dark brown spots presents in the ventral abdomen of *R. marabaensis* sp. n. does not appear in *R. prolixus* or *R. robustus*. The ventral connective is also distinct among the three species: the black spots are smaller and, on the sixth segment, much smaller in *R. marabaensis* sp. n. The apex of the endosoma of male genitalia of *R. marabaensis* sp. n. was found to be long and straight, in *R. prolixus*, the apex is long and convex, and in *R. robustus* it is shorter, wide, and convex. The posterior surface and the ventral surface of the ninth and tenth segments of external female genitalia are distinct in the three species. *R. marabaensis* sp. n. eggs possess chorion rims, whereas those of *R. prolixus* and *R. robustus* do not (Figs 1, 3, 5, 6, 7, 10, 11, 12, 13).

Description. Measurements of 15 females and 15 males of *R. marabaensis* sp. n., *R. prolixus,* and *R. robustus* are detailed in the Table 1.

Head with apex (central longitudinal dorsal portion), which is elevated, straw yellow, and keel shaped. This keel-shaped section presents the same shape from the beginning of the clypeus to the posterior portion of the ocelli; thus, the border of the clypeus is visible around/from the gena and the jugum (1+1), which are located laterally. However, the gena begin before the beginning of the clypeus. Thus, the gena go toward the anteclypeus which are rounded. The species presents crystalline ocelli and eyes with black and yel-

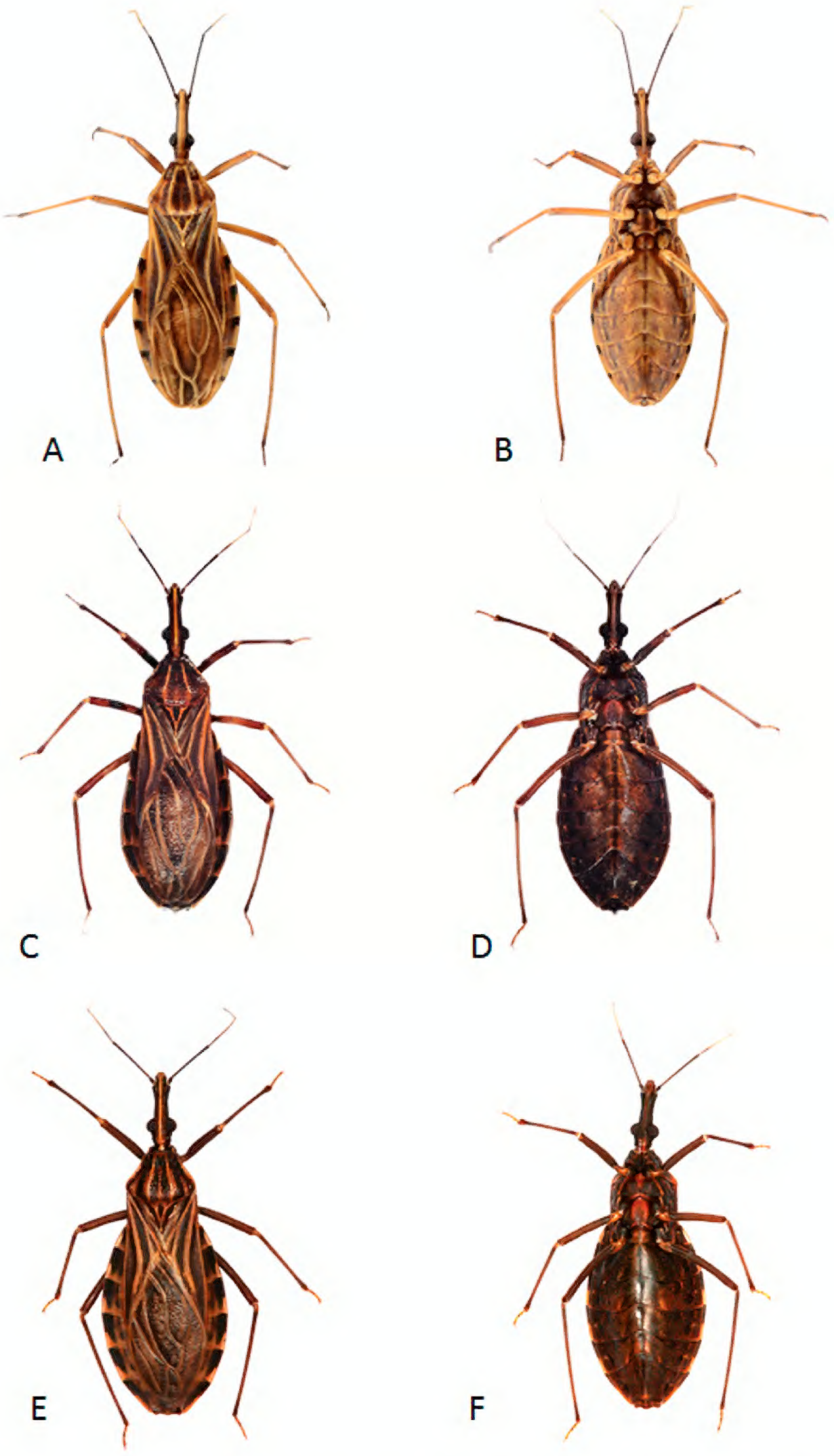

Figure 2. *R. marabaensis* sp. n. female (**A** dorsal side **B** ventral side); *R. prolixus* female (**C** dorsal side **D** ventral side); *R. robustus* female (**E** dorsal side **F** ventral side).

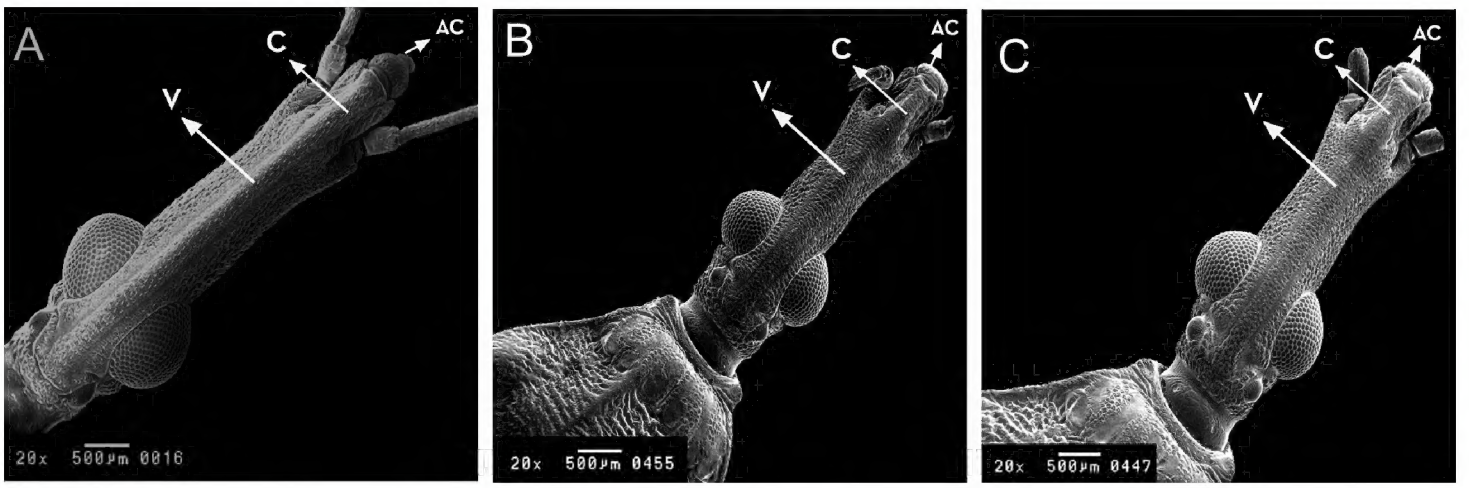

Figure 3. Head of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). V: vertice; C: clypeus; AC: anteclypeus.

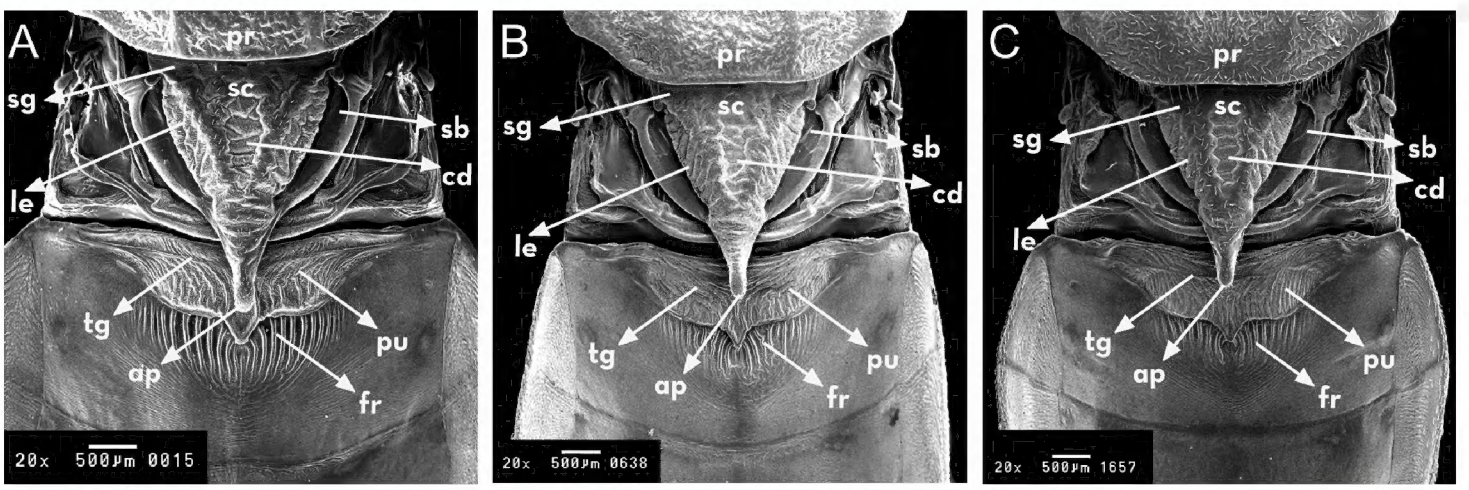

Figure 4. Escutellum and process of I urotergit of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). pr: pronotum; sc: escutelum; sb: semi- circular base; sg: glabrous space; cd: central depression; le: lateral edge; ap: apex of escutelum; pu: process of I urotergit; tg: transverse groove; fr: fringe.

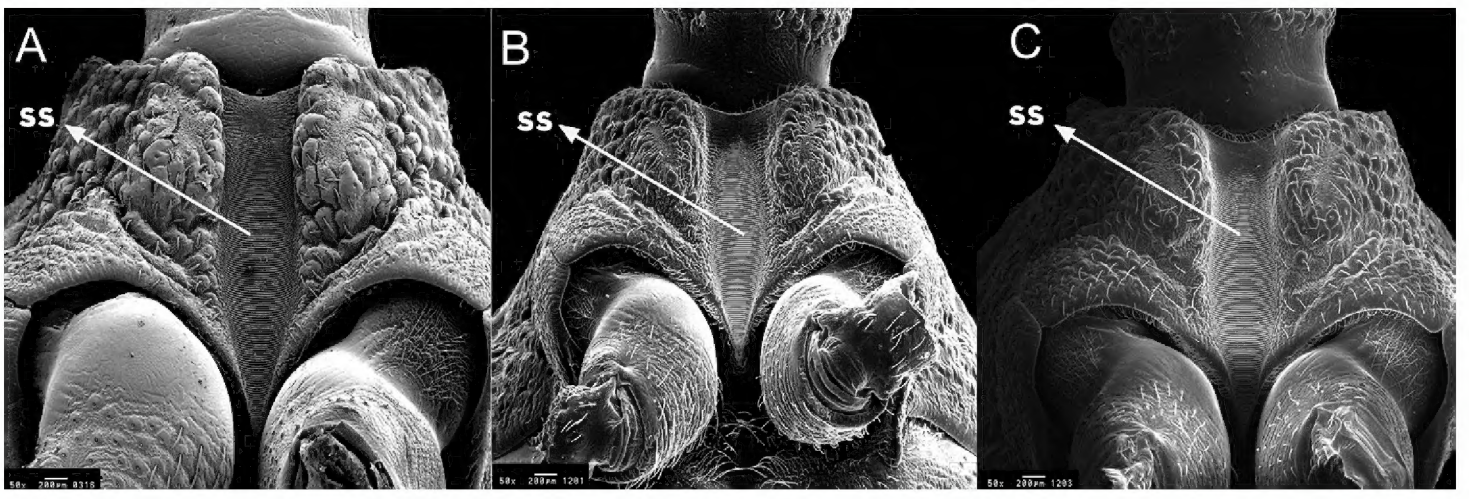

Figure 5. Thorax ventral of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). SS: Stridulatory sulcus.

low ommatidia. The first and second segments of the antennae are yellow, whereas the posterior two thirds of the third segment are white, and the fourth segment is completely white. The species presents a second antennal segment that is significantly larger than the others (10.3 times larger than the first; 1.65 times larger than the third, and 4.3 times larger than the fourth) (Table 1).

Table 1. Mean of measurement (mm) of 15 females and males of *R. marabaensis* sp. n., *R. prolixus*, and *R. robustus*.

	Male			Female		
	R. marabaensis	***R. prolixus***	***R. robustus***	***R. marabaensis***	***R. prolixus***	***R. robustus***
HL	**4.90** [a]	3.87 [b]	3.82 [c]	**5.32** [a]	3.90 [b]	4.06 [c]
IE	0.62 [a]	0.53 [b]	0.64 [a]	0.59 [a]	0.56 [b]	0.67 [c]
AO	2.21 [a]	2.05 [b]	2.23 [c]	3.04 [a]	2.26 [b]	2.38 [c]
PO	0.98 [a]	0.92 [b]	0.72 [c]	1.06 [a]	0.77 [b]	0.78 [c]
DE	1.72 [a]	1.94 [b]	1.00 [c]	1.91 [a]	1.68 [b]	1.64 [c]
R1	0.97 [a]	0.55 [b]	0.91 [c]	0.93 [a]	0.57 [b]	0.97 [c]
R2	**3.87** [a]	3.25 [b]	3.02 [c]	**3.77** [a]	3.32 [b]	3.30 [c]
R3	0.87 [a]	0.33 [b]	0.92 [c]	0.77 [a]	0.39 [b]	0.96 [c]
TL	**20.41** [a]	19.98 [b]	20.20 [c]	**22.35** [a]	20.98 [b]	21.28 [c]
MWT	4.25 [a]	4.62 [b]	4.06 [c]	4.88 [a]	4.82 [b]	4.12 [c]
MWA	**6.22** [a]	5.93 [b]	6.03 [c]	**6.92** [a]	6.75 [b]	6.56 [c]
A1	0.48 [a]	0.38 [b]	0.37 [c]	0.45 [a]	0.37 [b]	0.38 [c]
A2	**4.72** [a]	3.04 [b]	3.28 [c]	**4.47** [a]	2.88 [b]	3.18 [c]
A3	2.68 [a]	2.25 [b]	2.32 [c]	3.05 [a]	1.94 [b]	2.41 [c]
A4	1.05 [a]	0.98 [b]	1.54 [c]	1.15 [a]	0.94 [b]	1.64 [c]

HL: head length; IE: inner distance between eyes; AO: anteocular distance; PO: postocular distance (excluding neck); DE: diameter of the eye; R1, R2 and R3: lengths of first, second and third rostral; TL: total length of the triatomine; MWT: maximum width of the thorax; MWA: maximum width of the abdomen; A1, A2, A3 and A4: 1st, 2st, 3st, e 4st antennal segments, respectively; a,b,c: Lower case letters indicate significant differences between specimens with Tukey's test: $p < 0,05$. Values in bold indicate the main findings.

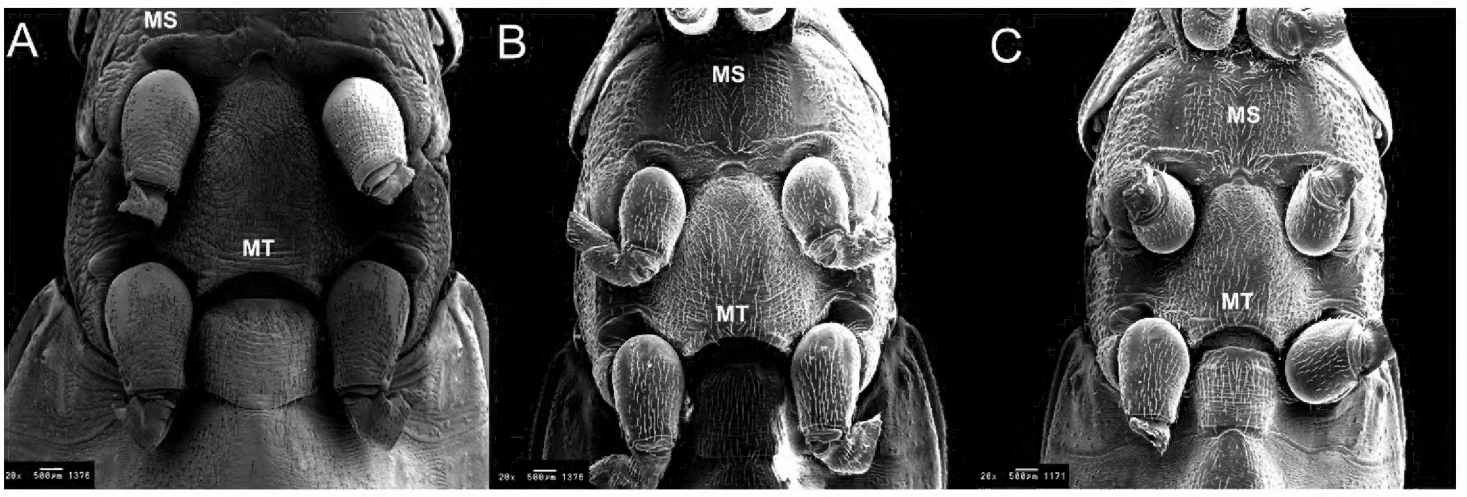

Figure 6. Thorax ventral of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). ms: mesosternum; mt: metasternum.

At the juncture between the neck and the thorax, there is a ring that is anteriorly black and posteriorly yellow; the anterolateral angles (1+1) are yellow. The dorsal thorax (pronotum) is shaped like a trapezoid and surrounded by a yellow carina. There are two yellow submedian carinae running lengthwise around the pronotum, from the anterior portion to the posterior one. The submedian carina border three anterior

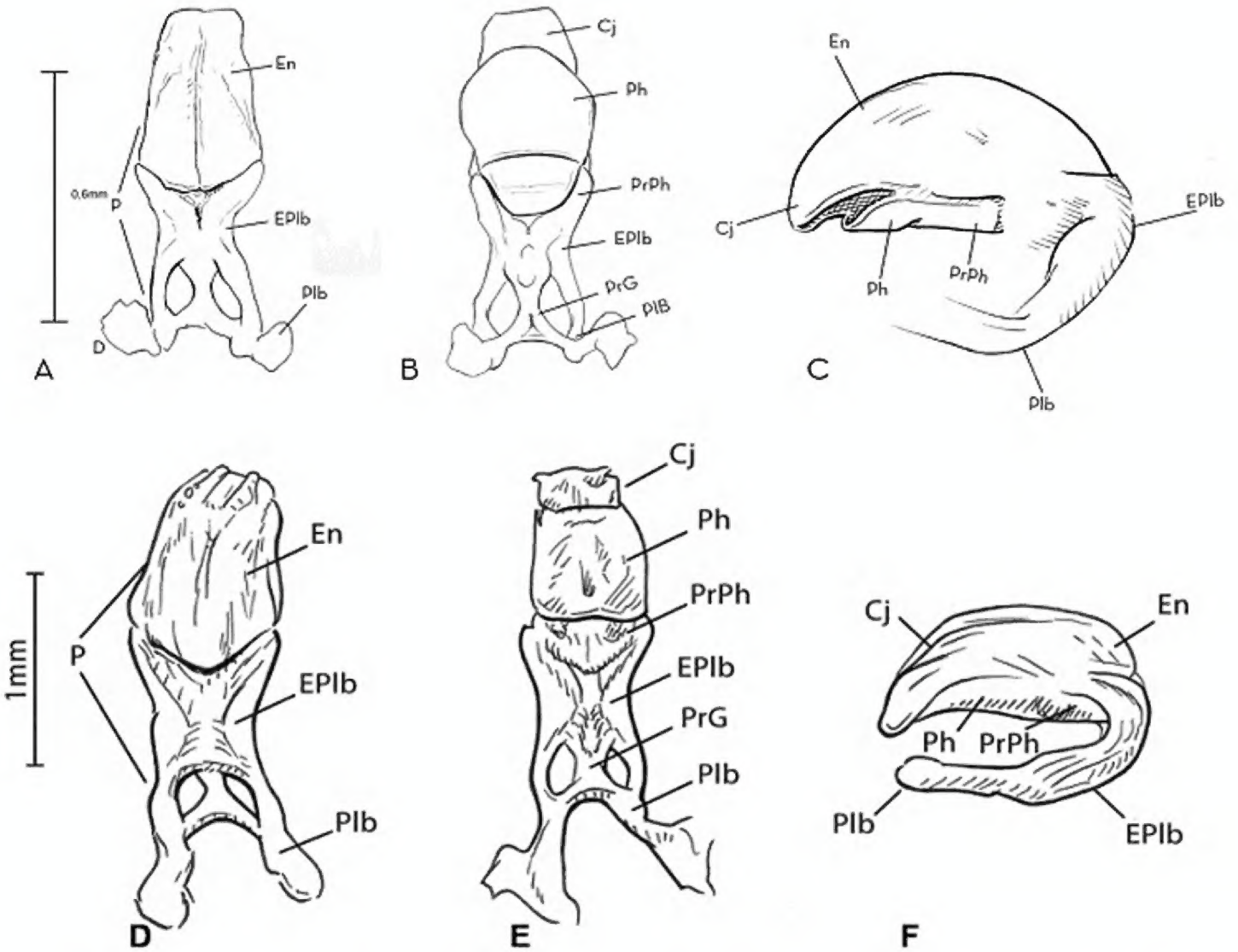

Figure 7. Phallus of *R. marabaensis* sp. n. (**A** dorsal view **B** ventral view **C** lateral view) and *R. robustus* (**D** dorsal view **E** ventral view **F** lateral view). Cj: conjunctive; En: endosome; EPlb; median extencion of basal plate; P: phallus; Plb: basal plate; PrG: gonopore process; PrPh: phallossoma process; Ph: phallosoma.

lobes, each of which has a set of black spots, and three posterior lobes with two parallel black stripes on each that are connected to the set of black spots on the anterior lobes. The triangular scutellum is marked laterally and is very clear because of its black color. The internal portion is also triangular and yellow, and it is bordered by thick and obvious carina. When the wings are removed, the posterior portion (tip) of the scutellum covers 2/3 of the I urotergite process (Figs 4, 5).

From the ventral surface of the thorax, a prosternum with deep, well-defined stridulatory black sulcus is visible; in the posterior portion, it takes on a funnel shape and ends as a tip between the anterior pair of legs (Fig. 5). The mesosternum has two elevated black areas that are separated by a yellow depression. The border between the mesosternum and the metasternum is formed by a set of three elevated and prominent carinae. The two lateral carinae are black, and the central carina is yellow. These three carinae are curved backward. The central carina, which is elevated and prominent, possesses a semicircular depression in the central portion at the border with the metasternum. The metasternum is slightly rectangular in shape. The central portion is black and outlined by two yellow carinae (Fig. 6).

The legs have an overall yellowish tone. The coxae have yellow and black spots; the trochanters are yellow and do not have spots; the femurs are yellow with black spots running lengthwise; the tibias are yellow except for the posterior sixth segment, which continues the black pattern of the tarsi (Fig. 2).

The ventral surface of the abdomen is predominantly yellow, with three sets of black stripes: one on the central longitudinal portion and (2+2) on the side portions above the connectives (Fig. 2). The first abdominal segment has a longitudinal black spot between the two larger yellow spots. The second, third, fourth, and fifth ventral abdominal segments possess (1+1) curved sets of dark brown spots. These spots begin at the anterior dividing line and extend diagonally along the central portion of the segments (Fig. 2). The dorsal connective includes yellow and black spots that cover half of each segment. They are wide in the anterior portion and become thinner in the inner posterior portion. The black spot of the connective of the sixth segment is smaller than those of the fifth, fourth, third, and second segments (Fig. 2). The first tergite, which is visible when the wings are removed, is essentially formed by two parts. The anterior portion has a striated cuticula that contrasts with the surrounding smooth cuticula and which is triangular in shape on its upper level. It possesses a clearly defined transverse sulcus. The second portion is posterior to the first and is at a lower level. It consists of a set of transverse and straight fringes (Fig. 5).

When the male genitalia is seen from the dorsal surface, it is clear that the phallus (P) is formed by an endosoma (En), by the median extension of the basal plate (EPlb), and by the basal piece itself (Plb). When seen from the ventral surface, the phallus (P) is formed by the conjunctiva (Cj), the phallosoma (ph), the phallosoma process (PrPh), the median extension of the basal piece (EPlb), the gonopore process (PrG), and the basal piece (Plb). When seen from the lateral surface, the phallus is formed by the conjunctiva (Cj), the endosoma (En), the median extension of the basal piece (EPlb), the basal piece itself (Plb), the phallosoma (Ph), and the phallosoma process (PrPh) (Fig. 8).

The dorsal surface of the external female genitalia was examined using scanning electron microscopy (SEM), which showed that the seventh segment is separated from the eighth segment by a slightly irregular line and forms (1+1) triangular tips at the border between the connective and the eighth segment. The eighth segment is trapezoid shaped. The ninth segment appears as a protrusion. The tenth segment appears as a small curve in the central portion where it delimits with the eighth segment (Fig. 9)

From the posterior surface, (1+1) appendages can be seen on the border between the eighth and ninth segments. The tenth segment is semicircular in shape with a pronounced central slit in the shape of an upside-down V and with (1+1) protrusions at the posterior edge of the gonocoxite 8. Display is also a (1+1) gonocoxite 8 and a (1+1) gonapophysis 8 (Fig. 10).

From the ventral surface, the lateral portions of the line that divides the seventh segment and the gonocoxites 8 and the gonapophysis 8 are curved, and the line then forms small (1+1) ascending curves and a slight depression in the central portion. The ninth segment forms (1+1) lateral flaps at the border with the tenth segment and presents transverse slits at the sub-intermediate position (1+1). The transverse slits then

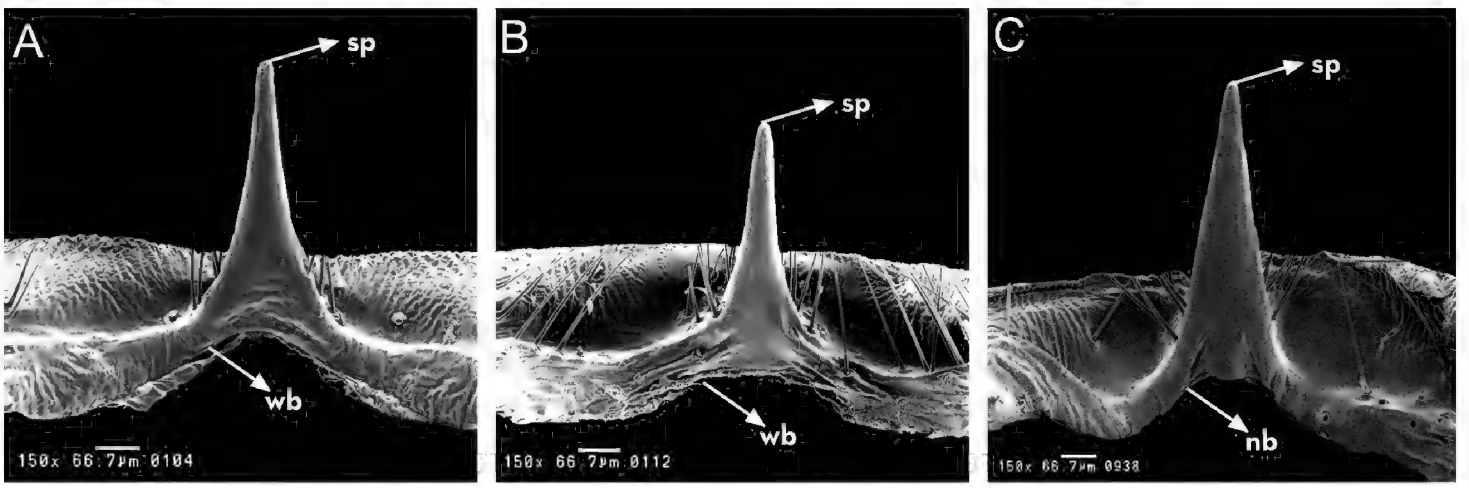

Figure 8. Median process of the pygophore of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). gp: gross point: nb: narrow triangular base; sp: slender point; wb: wide triangular base.

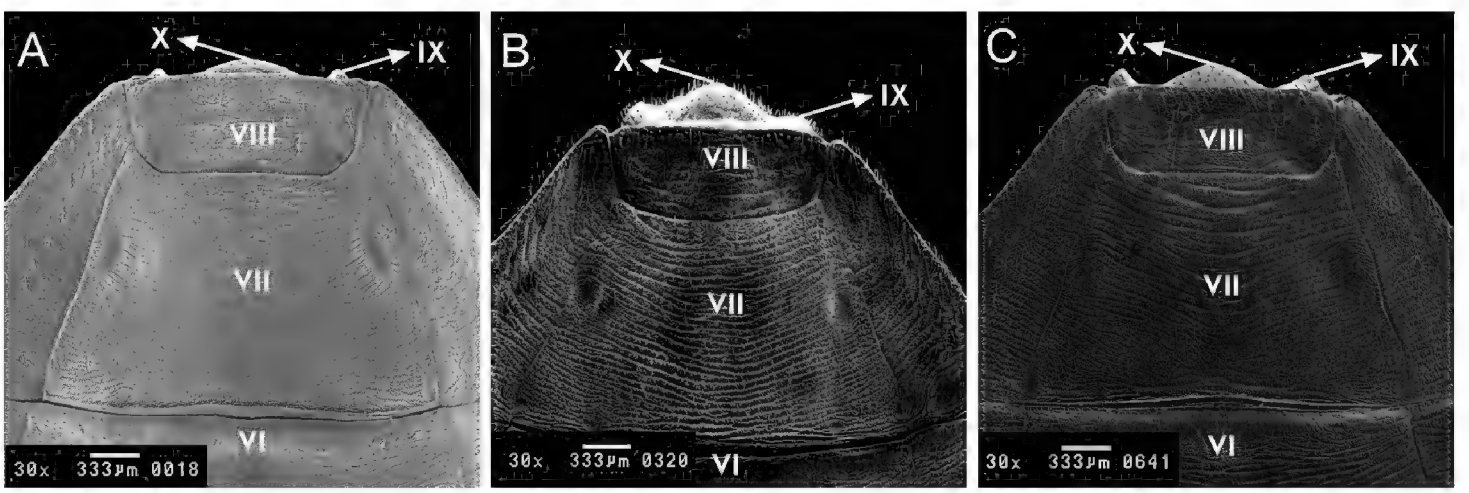

Figure 9. Female external genitalia, dorsal side of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). VI, VII, VIII, IX, X, tergites.

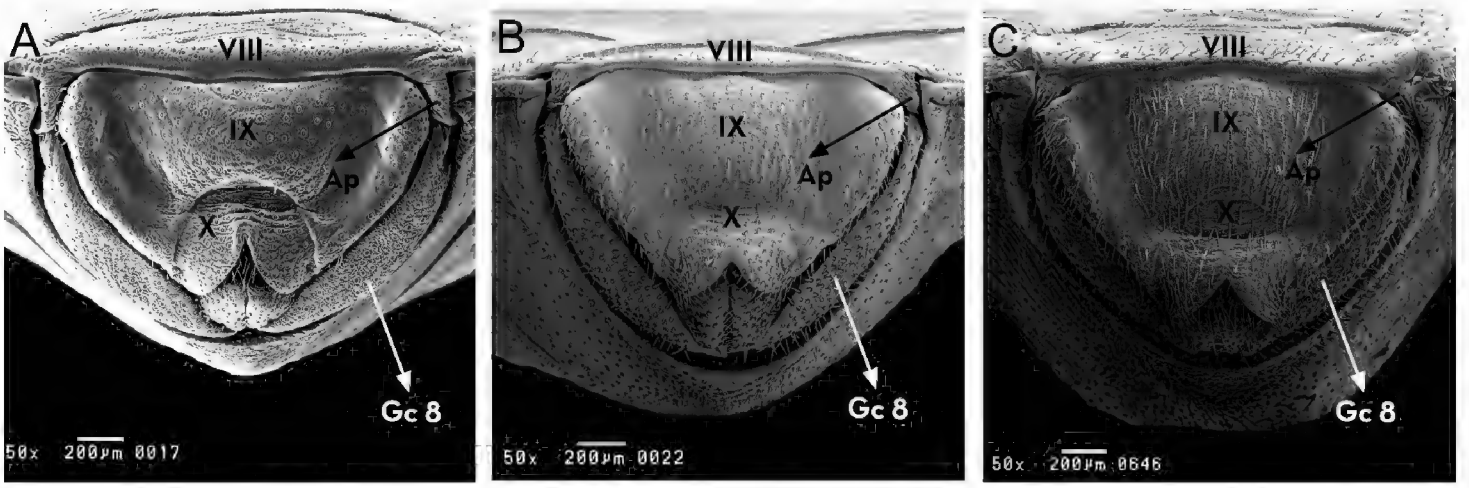

Figure 10. Female external genitalia, posterior side of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). Ap: appendices; Gc 8: gonocoxite VIII; Gp 8: gonaphofyse VIII; VII, VIII, IX: tergites; X: segment.

form into two triangles, whose tips are separated in the central portion. The tenth segment is the outer edge of the external female genitalia and is presented as a narrow curved and convex band (Fig. 11).

The egg shells measure 1.59 mm in length and 0.71 mm in width. They present prominent collar and chorial rim (Fig. 12 and Table 2). The exochorion cells are clear-

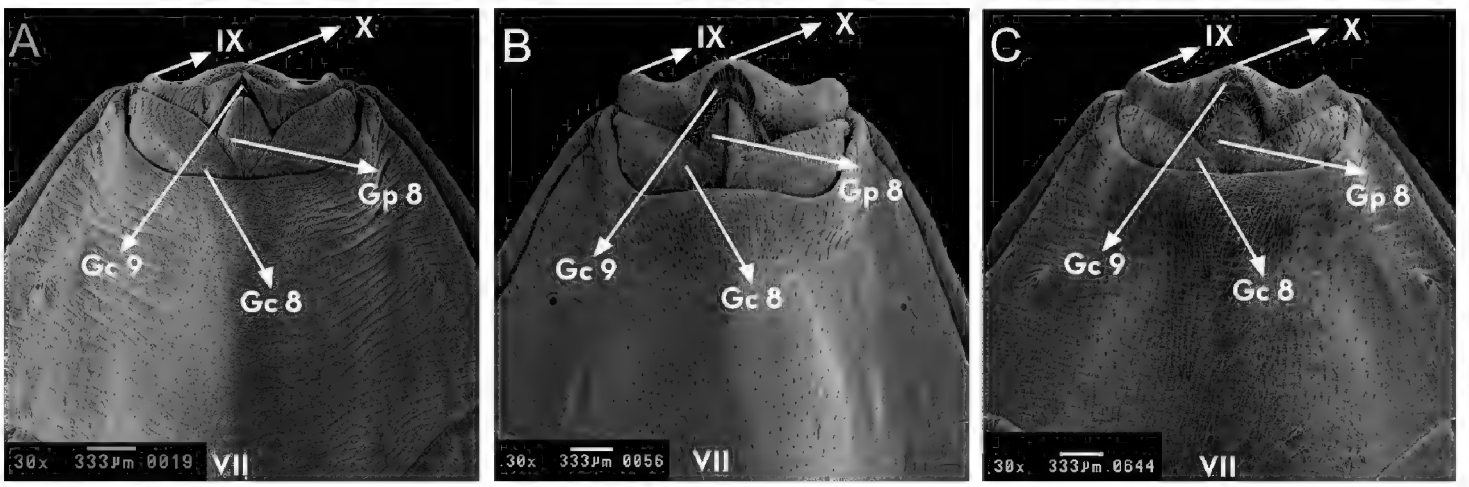

Figure 11. Female external genitalia, ventral side of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). Gc 8: gonocoxite VIII; Gc 9: gonocoxite IX; Gp 8: gonapofhyse VIII; VII, IX: esternites; X: segment.

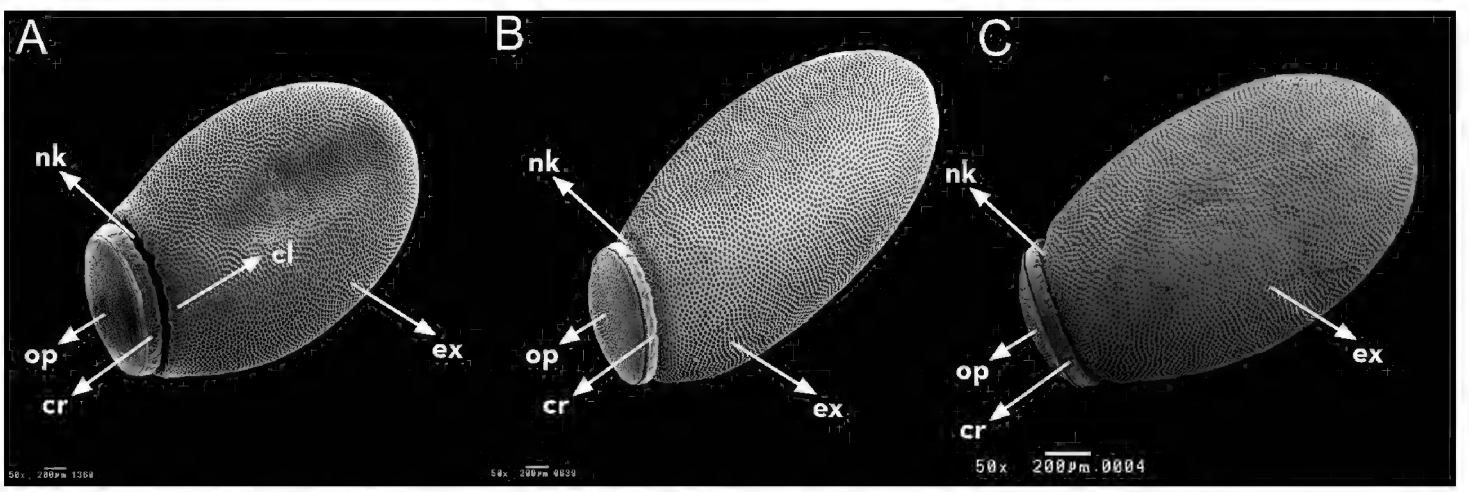

Figure 12. Eggs general vision of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). cl: colar; cr: chorial rim; ex: exochorium; nk: neck; op: operculum.

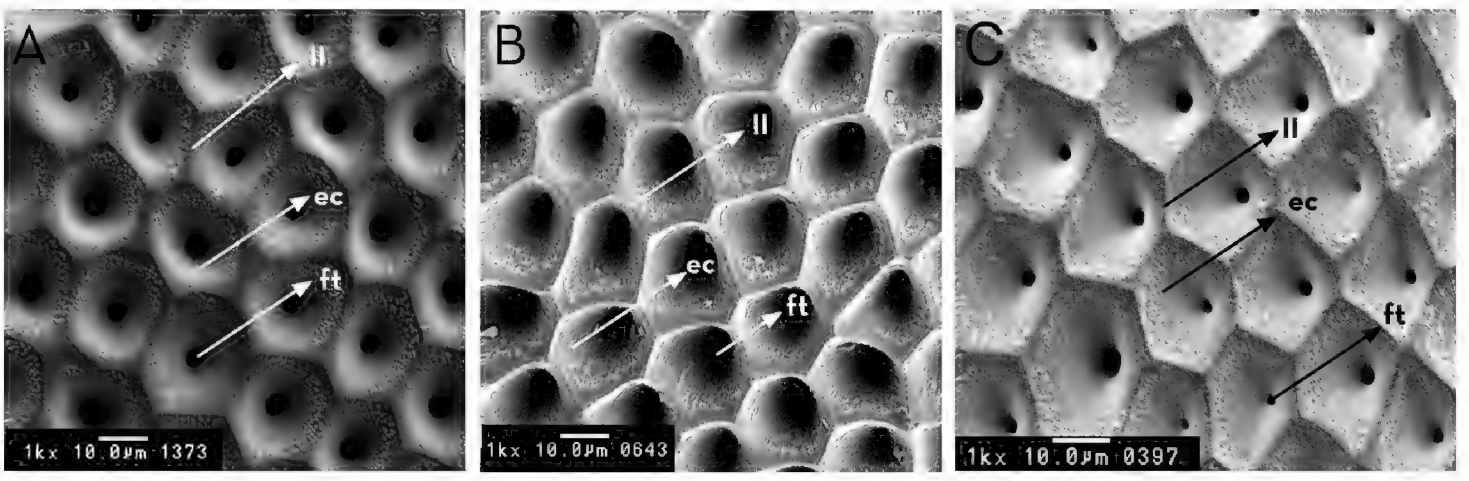

Figure 13. Egg exochorium detail of *R. marabaensis* sp. n. (**A**), *R. prolixus* (**B**), *R. robustus* (**C**). ec: exochorium cell; ft: follicular tubes; ll: limiting line.

Table 2. Mean of measurements (mm) of 13 eggs of *R. marabaensis* sp. n., *R. prolixus*, and *R. robustus*.

Measurement	*R. marabaensis*	*R. prolixus*	*R. robustus*
L (mm)	1.54 ± 0.04 [a]	1.73 ± 0.02 [b]	1.61 ± 0.04 [c]
W (mm)	0.87 ± 0.01 [a]	0.71 ± 0.06 [b]	0.93 ± 0.01 [c]
Oo (mm)	0.49 ± 0.01 [a]	0.67 ± 0.01 [b]	0.73 ± 0.01 [c]

L: length (40×); L: width (40×); Oo: opening of operculum (80×); a,b,c: Lower case letters indicate significant differences between specimens with Tukey's test: $p < 0,05$.

ly demarcated, with internal granulations organized into a circle. The follicular tubes of each exochorion cell do not differ in diameter (Fig. 13).

The molecular study shown the same haplotype for the find sequences of the cyt-b (693 bp) and the evaluation by BLAST have shown that *R. marabaensis* sp. n. is closely related to *R. robustus* and *R. prolixus* (until 99% and 94% of identity, respectively).

Discussion

In epidemiological terms (i.e., considering the role of the species as vectors of *T. cruzi*), the three main Triatominae genera are *Panstrongylus*, *Rhodnius*, and *Triatoma*. Distinguishing among these three genera is not difficult because they can be characterized macroscopically based on the format of the head and the position of the antenniferous tubercle, as described by Pinto (1931). However, distinguishing among *Rhodnius* species requires a more detailed examination through optical microscopy. The difficulty in identifying *Rhodnius* species was first noted by Neiva and Pinto (1923). At the time, there were five known species: *R. nasutus*, *R. prolixus*, *R. pictipes*, *R. brethesi*, and *R. domesticus* (Lent and Wygodzinsky 1979). Including this description of *R. marabaensis* sp. n., there are currently 20 species (Rosa et al 2012, Abad Franch 2013); therefore, specific distinction is even more difficult and requires more characteristics to be considered. As a result, the characterization of *R. marabaensis* sp. n. is discussed comparatively using *R. prolixus* and *R. robustus*. *R. marabaensis* is closely related to *R. robustus* and *R. prolixus* (until 99% and 94% of identity, respectively). In view of this and of the fact that all three species occur in Northern Brazil (Galvão 2014), *R. robustus* and *R. prolixus* were morphologically examined and compared in more detail (Table 3).

Rhodnius marabaensis sp. n. is predominantly yellow, whereas *R. prolixus* is black and *R. robustus* is brown. The legs of the three species are also consistent in these color schemes (Fig. 2). Another very clear characteristic of *R. marabaensis* sp. n. is that it is larger than *R. prolixus* and *R. robustus* (Figs 3–5 and Table 1).

The head of *R. marabaensis* sp. n. has four very clear characteristics that help to distinguish it from the others:

1) It possesses a keel-shaped longitudinal dorsal portion (apex) running from the clypeus to the ocelli. This feature is not accentuated in *R. prolixus* or *R. robustus* (Fig. 3);
2) It possesses a rounded anteclypeus. In *R. prolixus* and *R. robustus*, the anteclypeus is flat (Fig. 3);
3) It possesses an indistinguishable clypeus. In *R. prolixus*, it is narrow in the anterior portion and wide in the posterior one. In *R. robustus* it is wide in the anterior portion, narrow in the medial portion, and wide in the posterior one (Fig. 3);
4) The second segment of the antenna is significantly larger than the other three, with the following length ratio among the four antennal segments: 2nd > 3rd > 4th > 1st. This relative length pattern of the four antennal segments is the same as the pattern observed by Rosa et al. (2010) in *R. neglectus* and *R. prolixus* adults; however, the

Table 3. Triatominae species found in the Amazon region.

Species	Descriptors	Distribution	References
R. amazonicus	Almeida, Santos & Sposina, 1973	Brazil (Amazonas), French Guiana (Cacao, Saul)	Galvão 2014
R. barretti	Abad-Franch, Palomeque & Monteiro, 2013	Colombia (Puerto de Assís), Ecuador (Lagro Ágrio)	Abad-Franch et al. 2013
R. brethesi	Matta, 1919	Brazil (Amazonas), Venezuela (Aragua)	Galvão 2014
R. colombiensis	Mejia, Galvão, Jurberg, 2009	Colombia (Tolima)	Guhl et al. 2007
R. dalessandroi	Carcavallo & Barreto, 1976	Colombia (Meta)	Guhl et al. 2007
R. ecuadoriensis	Lent & Leon, 1958	Ecuador (Manabi, Eujias, Loja), Peru (Amazonas, Tumbis, Piura, Cojamarca, La libertad, Lambayeque e San Martin)	Galvão et al. 2003
R. neivai	Lent, 1953	Colombia (Cesar), Venezuela (Lara, Falcón, Zulia)	Galvão et al. 2003
R. milesi	Carcavallo et al., 2001	Brazil (Pará)	Galvão 2014
R. montenegrensis	Rosa et al., 2012	Brazil (Rondônia, Acre)	Galvão 2014
R. pallescens	Barber, 1932	Colombia (Bolívar, Sucre)	Arboleda et al. 2009
R. paraensis	Sherlock, Guitton & Miles, 1977	Brazil (Amazonas,Pará), French Guiana	Galvão 2014
R. pictipes	Stal, 1872	Brazil, French Guiana, Colombia, Peru, Suriname, Venezuela	Galvão 2014
R. prolixus	Stal, 1859	Brazil, Bolivia, Colombia, Guatemala	Galvão et al. 2003
R. robustus	Larousse, 1927	Brazil, Bolivia, Colombia, Venezuela, French Guiana	Galvão 2014
R. stali	Lent, Jurberg & Galvão, 1993	Brazil (Mato Grosso do Sul, Acre), Bolivia (Santa Cruz, La Paz)	Matias et al. 2003

Source: Galvão 2014; Galvão et al. 2003; Matias et al. 2003.

differences in size between the largest and the smallest segments are distinct: the second antennal segment of *R. marabaensis* sp. n. is 10.3 times larger than the first; in *R. prolixus*, it is 6.2 times larger, and in *R. robustus* it is 8.3 times larger (Table 1).

For the description of *R. marabaensis* sp. n. five features of the thorax allow for its distinction:

1) The scutellum is larger and includes two prominent internal lateral carinae. It is therefore distinct from *R. prolixus* and *R. robustus*, which present smaller scutellum and whose carinae are not pronounced (Fig. 4);
2) The first urotergite has a pronounced transverse groove and inferior fringe consisting of long and straight filaments. In *R. prolixus*, the transverse groove does not appear and the fringe possesses short and irregular filaments. On the other hand, in *R. robustus* the transverse groove is not accentuated, and the fringe possesses short and straight filaments, as shown in (Fig. 5).

3) It possesses a longer and more clearly shaped stridulatory sulcus relative to those of *R. prolixus* and *R. robustus* (Fig. 5). This specific differentiation among these three *Rhodnius* species adds to the observations by Lent and Wygodzinsky (1979), who verified that the stridulatory sulcus in six species from different genera presented characteristics that allowed for species identification;
4) The transverse carinae that border the mesosternum and the metasternum differ among the three species. In *R. marabaensis* sp. n., they are elevated and prominent, and possess convex curvature in the central portion. In *R. prolixus*, they are less elevated and prominent, and in *R. robustus* they are interrupted in the central portion (Fig. 6);
5) The metasternum is slightly rectangular in shape in *R. marabaensis* sp. n., whereas in *R. prolixus* and *R. robustus* they are slightly triangular (Fig. 6).

From the view of the ventral surface, the abdomen of *R. marabaensis* sp. n. is distinct because of its yellow colouring; this area is brown in *R. prolixus* and black in *R. robustus* (Fig. 11). The set of dark brown spots does not appear in *R. prolixus* or *R. robustus*, and the ventral connective is also distinct among the three species: the black spots are smaller and, on the sixth segment, much smaller in *R. marabaensis* sp. n. (Fig. 2).

When the male genitalia was examined from the dorsal surface, the apex of the endosoma of *R. marabaensis* sp. n. was found to be long and straight; in *R. prolixus*, the apex is long and convex, and in *R. robustus* it is shorter, wide, and convex (Lent and Jurberg, 1969) (Fig. 7). The analysis also revealed that the two final portions of the basal piece are turned to the side in *R. marabaensis* sp. n., whereas in *R. robustus* they face the posterior region. From the ventral surface, the phallosoma of *R. marabaensis* sp. n. is rounded, whereas in *R. prolixus* the anterior portion is convex and in *R. robustus* it is square (Fig. 7). The pygophore of *R. marabaensis* sp. n., *R. prolixus*, and *R. robustus* are all in the shape of an isosceles triangle. However, the sides of the pygophores are straight in *R. robustus* and curved in *R. prolixus* and *R. marabaensis* sp. n. The pygophore of *R. marabaensis* sp. n. is also larger at the tip, thicker, and circular (Fig. 8).

The external female genitalia was also considered. From the dorsal surface, *R. marabaensis* sp. n. and *R. robustus* show no differences; however, the format of the ninth segment of the dorsal surface in *R. prolixus* presents different characteristics (Fig. 9). The posterior surface and the ventral surface of the ninth and tenth segments are distinct in the three species (Fig. 10). These characteristics are also distinct from 12 other *Rhodnius* species, as described by Rosa et al. (2014).

Rhodnius marabaensis sp. n. eggs possess chorion rims, whereas those of *R. prolixus* and *R. robustus* do not. The diameter of the follicular tubes of the exochorion cells in *R. prolixus* are larger than those of *R. marabaensis* sp. n. and *R. robustus*; however, they are regular in *R. marabaensis* sp. n. and varied in *R. robustus* (Figs 12, 13 and Table 2).

The data obtained corroborate the status of *R. marabaensis* sp. n. as a new species and indicates that the use of morphology for the description of Triatominae species offers phenotypic information (morphological and morphometric) to define the status of species. It is also important to note that molecular analysis generates data that can

help phylogenetic relationships and taxonomic studies of Triatominae (Abad-Franch and Monteiro 2005, Justi et al. 2014). BLAST analysis of cyt-b sequence shows *R. marabaensis* sp. n. as closely related to *R. robustus* and *R. prolixus*, so this new species must be included in the *R. prolixus* complex (Carcavallo et al. 2000). Complementary approaches using molecular data must be encouraged to establish the phylogenetic placement of this new species based on the evaluation of other gene fragments and a more robust assessment.

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